US Army Corps of Engineers

AQUATIC PLANT CONTROL RESEARCH PROGRAM

TECHNICAL REPORT A-88-7

BIOLOGICAL CONTROL OF WATERHYACINTH IN THE CALIFORNIA DELTA

by

R. Michael Stewart, Alfred F. Cofrancesco, Jr

Environmental Laboratory

DEPARTMENT OF THE ARMY
Waterways Experiment Station, Corps of Engineers
PO Box 631, Vicksburg, Mississippi 39180-0631

and

Larry G. Bezark

Biocontrol Services Program
California Department of Food and Agriculture
Sacramento, California 95814

June 1988
Final Report

Prepared for US Army Engineer District, Sacramento
Sacramento, California 95814-4794

and

California Department of Boating and Waterways
Sacramento, California 95814

Destroy this report when no longer needed. Do not return it to the originator.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

The contents of this report are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or approval of the use of such commercial products.

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Technical Report A-88-7	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION See reverse. / 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) See reverse.	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION See reverse. / 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Sacramento, CA 95814-4794; Sacramento, CA 95814	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Biological Control of Waterhyacinth in the California Delta

12. PERSONAL AUTHOR(S)
Stewart, R. Michael; Cofrancesco, Alfred F., Jr.; Bezark, Larry G.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final report	FROM _____ TO _____	June 1988	50

16. SUPPLEMENTARY NOTATION
Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Aquatic plant control; Insects; Biological control; Waterhyacinth; California Delta

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The purpose of this project was to transfer biocontrol technology developed for waterhyacinth control in the southeastern states to agencies responsible for waterhyacinth control in California. This technology centers on the use of three South American insect species [*Neochetina bruchi* Hustache and *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and *Sameodes albiguttalis* Warren (Lepidoptera:Pyralidae)] which effect natural control of waterhyacinth in its native range. The technology transfer consisted of (a) releasing the biocontrol agents at specific sites in California, (b) monitoring the establishment of these agents, (c) evaluating the control effectiveness by these agents, and (d) monitoring the natural dispersion by these agents.

(Continued)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code) / 22c. OFFICE SYMBOL

DD Form 1473, JUN 86 *Previous editions are obsolete.*

SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

6a. NAME OF PERFORMING ORGANIZATION (Continued).

USAEWES, Environmental Laboratory;
California Department of Food and Agriculture

6c. ADDRESS (Continued).

PO Box 631, Vicksburg, MS 39180-0631;
Sacramento, CA 95814

8a. NAME OF FUNDING/SPONSORING ORGANIZATION (Continued).

USAED, Sacramento;
California Department of Boating and Waterways

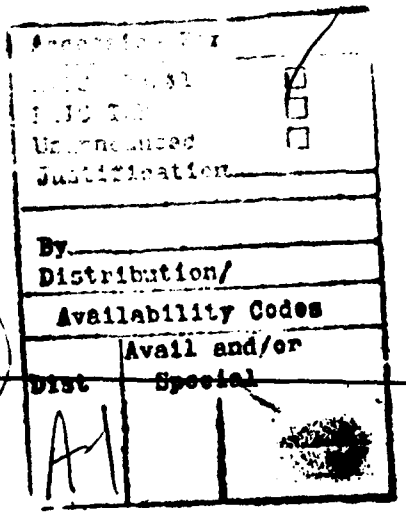
19. ABSTRACT (Continued).

Neochetina bruchi was first released in the Delta in July 1982; *N. eichhorniae* in April 1983; and *S. albiguttalis* in August 1983. Release efforts consisted of the following: *N. bruchi* and *S. albiguttalis* at the Old River Site; *N. eichhorniae* and *S. albiguttalis* at the White Slough Site; all three species at the Trapper Slough and Veale Tract sites. This release scenario was used to determine the effectiveness of different combinations of the biocontrol agents.

Population levels of *N. bruchi* were successfully established at the Old River and Veale Tract sites. Population levels necessary to effect widespread damage to the waterhyacinth population were observed at the Old River Site. *Neochetina eichhorniae* was successfully established at the White Slough and Veale Tract sites, but control effectiveness was not demonstrated. The establishment of *S. albiguttalis* was not confirmed at the four sites.

The establishment of new colonies of the biocontrol agents by natural dispersion from the release sites was not demonstrated. It was evident that the widespread effectiveness of chemical control in the Delta reduced the ability of the insects to disperse.

Recommendations for more effective use of biocontrol in the San Joaquin River System are presented. These recommendations center on the establishment of biocontrol agent populations in the upstream portions of the San Joaquin River System.



PREFACE

The transfer of biocontrol technology to the California Department of Boating and Waterways (CDBW) for the management of waterhyacinth in the Sacramento-San Joaquin Delta is described in this report. This technology was developed through the Aquatic Plant Control Research Program (APCRP), which is sponsored by Headquarters, US Army Corps of Engineers (HQUSACE). The APCRP is managed by the US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. Technical Monitor for HQUSACE was Mr. Carl Brown. This project was funded by the CDBW through the US Army Engineer District, Sacramento (SPK).

This report was prepared by Mr. R. Michael Stewart and Dr. Alfred F. Cofrancesco, Jr., Wetlands and Terrestrial Habitat Group (WTHG), Environmental Resources Division (ERD), Environmental Laboratory (EL), WES, and Mr. Larry G. Bezark, Biocontrol Services Program (BCSP), California Department of Food and Agriculture, Sacramento, California. Principal investigators for the project were Mr. Edwin A. Theriot, Dr. Cofrancesco, and Mr. Stewart, WTHG.

The field research and data analyses were performed by Messrs. Stewart and Bezark, and by Dr. Cofrancesco, Mr. Harvey L. Jones, and Mses. Ramona H. Warren, Patricia A. Miller, WTHG, and Kathleen A. Casanave and Helen Yee, BCSP.

The screening of biocontrol agents prior to field releases was performed by Drs. Lloyd Andres and Arvin Krueger, US Department of Agriculture, Albany, California, and by Mr. Bezark, BCSP. Dr. Andres also assisted in some of the initial field releases of *Neochetina bruchi*.

The greenhouse colony of *Sameodes albiguttalis* in Sacramento was maintained by Mses. Cassanave and Yee. Special assistance during boat surveys was provided by Messrs. Ron Mason and Charles Ellenberg, both of Bryte Yard Facility, SPK.

The project was monitored by Messrs. Larry Thomas and Bill Satow, CDBW, and Mr. Keith Steele, SPK. The research was conducted under the direct supervision of Dr. Hanley K. Smith, Chief, WTHG, and under the general supervision of Dr. Conrad J. Kirby, Chief, ERD, and Dr. John Harrison, Chief, EL. Mr. J. L. Decell was Program Manager, APCRP.

Commander and Director of WES was COL Dwayne G. Lee, CE. Technical Director was Dr. Robert W. Whalin.

This report should be cited as follows:

Stewart, R. Michael, Cofrancesco, Alfred F., Jr., and Bezark, Larry G. 1988. "Biological Control of Waterhyacinth in the California Delta," Technical Report A-88-7 , US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

CONTENTS

BIOLOGICAL CONTROL OF WATERHYACINTH IN THE CALIFORNIA DELTA

PART I: INTRODUCTION

Background

1. The California Delta, located at the confluence of the Sacramento and San Joaquin rivers, includes a complex network of rivers, sloughs, and man-made channels. The majority of these waterways are leveed, and adjacent lands have been drained for intensive agricultural usage. Because of low amounts of precipitation during the growing season, agricultural irrigation places a heavy demand on the waterways. Additionally, major municipalities throughout California consume vast quantities of Delta water. Delta waterways are also a favorite recreational resource, and demand for their use supports over one hundred inland marinas and related businesses within the Delta.

2. Waterhyacinth [*Eichhornia crassipes* (Mart.)] Solms, native to South America, has been rated the eighth most important weed species in the world (Holm et al. 1977). The ability of waterhyacinth to quickly infest areas in the southeastern United States has long been recognized (Penfound and Earle 1948). Waterhyacinth infestations can impede navigation, disrupt water flow, increase water loss through evapotranspiration, increase habitat for disease vectors, and disrupt ecological balances affecting freshwater fisheries and wildlife.

3. The earliest documented account of waterhyacinth in California (Yolo County) is a 1904 herbarium specimen at the University of California, Berkeley. A historical account of the weed's dispersal in northern California is provided by Bock (1970). Several populations of waterhyacinth were located in the upstream portions of the San Joaquin River System. Though waterhyacinth was not established in the Delta at the time, Bock indicated that the presence of the upstream populations posed a threat to the Delta waterways.

4. By the mid-70's waterhyacinth had become established in the Delta. By 1977, the US Bureau of Reclamation routinely used an existing debris barrier and removal system for the mechanical removal of waterhyacinth at the Clifton Court Forebay pumping station on Old River to prevent disruption of

the water supply. By September 1981, over 4,000,000 sq m of Delta waterways were estimated to be in need of waterhyacinth control to maintain normal boating and other water-related activities.

5. To prevent continued expansion of the waterhyacinth infestations in the Delta, the 1982 California legislature designated the California Department of Boating and Waterways (CDBW) the lead agency responsible for waterhyacinth control in the Delta. Through this authority, the CDBW requested assistance from the US Army Corps of Engineers' Aquatic Plant Control Research Program (APCRP) to develop a waterhyacinth management plan for the Delta.

6. After preliminary evaluation of the available data, the APCRP recommended that three general categories of control technology be implemented immediately in the Delta. Chemical control was recommended to provide immediate relief from waterhyacinth infestations in high-use areas. Because chemical control involves the placement of herbicides either directly into the water or directly on the plant for the purpose of effective control, certain regulatory requirements often limit the use of this control technique.

7. Mechanical control techniques were recommended in high-use areas where herbicide application was inappropriate. Mechanical control systems recommended for the Delta included: (a) boom assemblies to prevent blockage of launches and berths at marinas, (b) pusher boats and conveyors to remove small, isolated infestations, (c) small capacity harvesters in main waterways, and (d) gridded fences and booms around water intake structures.

8. Biological control was the third technology recommended by the APCRP. In comparison to chemical and mechanical control, biological control is not useful in areas requiring immediate relief. As a long-term control technology, however, biological control has been used successfully in southeastern states to provide control in areas where chemical and mechanical techniques were not practical. The rationale for using biocontrol technology for waterhyacinth is discussed by Sanders, Theriot, and Perfetti (1985). Advantages include low application costs, limited maintenance, and limited environmental risk.

9. The CDBW accepted the recommendations of the APCRP and implemented an operational chemical control program in 1982. At the request of the CDBW, the US Army Engineer Waterways Experiment Station (WES) assumed the lead role in the biological control research effort. The WES researchers were assisted

during implementation of the effort by the Biocontrol Services Program (BCSP), California Department of Food and Agriculture.

Purpose and Objectives of the Project

10. This project was undertaken by WES to transfer existing biocontrol technology developed by the APCRP in the Southeast to the agencies responsible for waterhyacinth control in the California Delta and to gain valuable research data on the effectiveness of biocontrol agent combinations. Primary objectives of the project were as follows:

a. To establish founder colonies of the biocontrol agents in the Delta and in upstream portions of the San Joaquin River System.

b. To evaluate the control effectiveness of different combinations of biocontrol agents within the established sites.

c. To monitor natural dispersion of the biocontrol agents from the founder colonies into adjacent waterhyacinth infestations.

Scope and Content of Report

11. This report documents the transfer of biocontrol technology of waterhyacinth to the California Delta, and presents information on each component of the project separately. Part II provides information on the taxonomy and life history of the three biocontrol agents. This section also describes the characteristic damage of each agent to waterhyacinth. Additionally, Part II includes information on collection of the agents in southeastern states, and their subsequent shipment to California. Part III describes the results of efforts to establish founder colonies of the biocontrol agents. Also included in this section is the evaluation of the control effectiveness of the biocontrol agents at the field sites. Part IV describes the efforts to monitor natural dispersion of the biocontrol agents from the field sites. Part V provides a summary of the results and lists the overall conclusions of the project.

PART II: THE BIOCONTROL AGENTS

12. Investigations for biocontrol agents of waterhyacinth were initiated in the 1960's in South America (DeLoach 1976; DeLoach and Cordo, 1976a, b, 1978; Cordo and DeLoach 1978; Perkins and Maddox 1976). These searches resulted in the introduction of three insect species into Florida in the 1970's, including two weevil species, *Neochetina eichhorniae* Warner and *N. bruchi* Hustache, and the pyralid moth, *Sameodes albiguttalis* Warren (Perkins 1973, Perkins and Maddox 1976, Center 1981a, b, 1982a). The weevils were subsequently released in Louisiana (Manning 1979) and in Texas (Cofrancesco in preparation). The efficacy of these control agents has been evaluated in Florida (Center, Durden, and Carman 1984), in Louisiana (Addor 1977; Goyer and Stark 1984; Sanders, Theriot, and Perfetti 1985, Sanders and Theriot 1986) and in Texas (Cofrancesco, in preparation).

Description and Life History

Waterhyacinth weevils

13. *Neochetina bruchi* Hustache and *N. eichhorniae* Warner both belong to the weevil tribe, Bagoini, and are similar in appearance. Adults (Figure 1a) are approximately 4 to 5 mm in length, are brownish to grey in color, and have yellowish hydrofuge scales around the bases of the legs. *Neochetina bruchi*, the chevroned waterhyacinth weevil, is best distinguished in the field by the presence of a light-tan chevron on the elytra. Other distinguishing taxonomic characters are provided by DeLoach (1975) and O'Brien (1976). Taxonomic keys are not available for immature stages of these weevils.

14. The biology of the two weevil species is also similar. Unless otherwise stated, the following information describes both species. Adult weevils are reclusive during daylight hours, but climb to the tops of waterhyacinth leaves at night to feed. Adults produce round feeding scars ca. 2 to 5 mm in diameter, mainly on the upper surface of the leaf blade (Figure 1b), but also along the petioles when population levels are high.

15. Adult females oviposit mainly by insertion of eggs into the petiolar tissues. The eggs are truncate (ca. 0.8 mm length, 0.6 mm width) and whitish in color. Eclosion normally occurs in 6 to 10 days.

a. Adult weevil on leaf surface

b. Adult feeding scars on leaf surface

c. Mature larva at base of leaf petiole

d. Pupal case, opened to show pupa, in root tissues

Figure 1. Life stages and typical adult feeding damage of *Neochetina* spp. observed during field studies

16. The grub-like larva (Figure 1c) is uniformly white with a light-brown head capsule. Developing larvae pass through three instars. As development proceeds, larvae display an internal migration toward the petiolar bases, where they do extensive damage to the plant rhizome and meristematic tissues. Larval development varies between 30 to 60 days, depending on temperature. Generally, *N. bruchi* develops more quickly than *N. eichhorniae*.

17. The pupal stage requires about 30 days and occurs underwater. The mature larva surrounds itself with an interwoven mass of root hairs and attaches itself to the root system of the plant (Figure 1d). Because successful completion of the pupal stage depends on the continued attachment of the pupal case to the root system, it is possible that the plant provides oxygen to the developing pupa.

Waterhyacinth moth

18. The Argentine waterhyacinth moth, *Sameodes albiguttalis* Warren, is a member of the family Pyralidae. Center (1981a) provides a detailed taxonomic description of this species.

19. The adult moths are approximately 20 mm in length and are brownish in color (Figure 2a). This brownish coloration is highly variable, but female moths are normally darker than males. Adults have vestigial mouthparts, and consequently do not feed. The life span of this stage is usually 4 to 6 days.

20. Females oviposit most of their eggs during the second night following emergence. Normal oviposition rates are 300 to 400 eggs per female. Eggs (Figure 2b) are often laid in damaged areas on the leaf blade of bulbous petioles. Eclosion requires about 4 to 5 days, but development is prolonged by low temperatures.

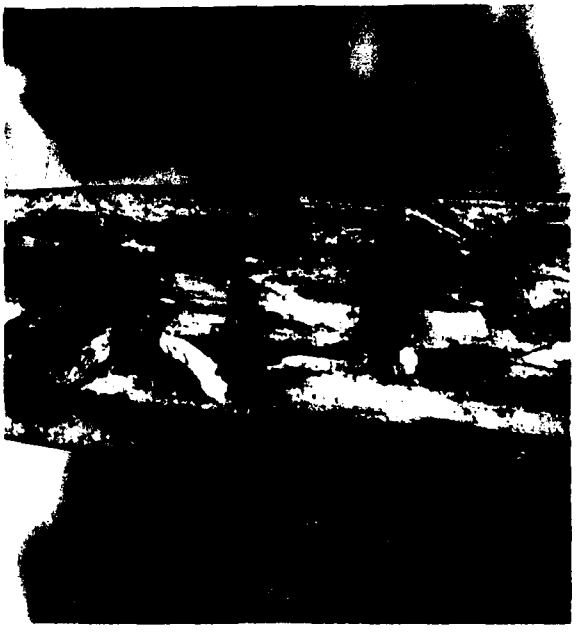
21. The first instar larva hatches and feeds for a brief period on the external surface of the leaf blade where it produces irregularly shaped scars. After several hours, the larva burrows into the petiole and feeds on internal tissues. During development through five instars, the larva continues feeding in the petiole and eventually migrates to the rhizome, where the fifth instar larva feeds extensively on meristematic tissues (Figure 2c).

22. The mature larva leaves the rhizome and reenters a waterhyacinth petiole where it excavates an internal pupal chamber. Immediately prior to pupation, the larva excavates an additional tunnel from the pupal chamber to the interior surface of the epidermal tissue. This produces a circular

a. Adult moth

b. Eggs laid on leaf, approximately 300-400 eggs per female

c. Mature larva in plant meristem



d. Pupal cases within plant petiole

Figure 2. Life stages of *Sameodes albiguttalis* observed during field studies

"hyaline window" through which the adult will emerge following pupation. Pupation occurs within a silken cocoon inside the pupal chamber (Figure 2d).

Collection and Screening of Biocontrol Agents

Waterhyacinth weevils

23. The source of *Neochetina* for this project was a field population adjacent to the Trinity River at Wallisville, Texas. Both species of *Neochetina* occur in this site, but *N. eichhorniae* is usually more abundant (Cofrancesco, in preparation).

24. Adult weevils were collected from heavily infested areas within the site by using sweep nets during the 2- to 4-hr period following dusk. Collection efforts usually provided 2,000 to 3,000 adults per night. Following collection, adults were placed on clean waterhyacinth tissue inside a cylindrical, waxed-cardboard container. These containers were placed in an ice chest, packed with newspaper and ice-packets, and shipped to the US Department of Agriculture (USDA) quarantine facility in Albany, California.

25. At the quarantine facility, the weevils were screened for pathogens/parasites and were separated to species. The segregated colonies were held on live waterhyacinth plants at the USDA facility until released at one of the Delta field sites.

Waterhyacinth moth

26. Sources of *S. albiguttalis* used in this project were from field populations in Florida and Louisiana. Pupae were collected from the field populations and shipped to the USDA facility in Albany.

27. At the USDA facility, pupae were placed in petri dishes and emerging adults were placed in mating pairs on fresh plant material to allow oviposition. After oviposition, adults were examined for pathogens. Offspring of pathogen-free adults were used to establish a resident colony. This colony was eventually moved to a BCSP greenhouse facility in Sacramento, California. Field releases were made from the BCSP colony by either releasing newly emerged larvae or by transplanting infested plants.

PART III: ESTABLISHMENT AND EFFECTIVENESS OF BIOCONTROL AGENTS

Overall Test Design

General

28. The initial efforts in this portion of the project consisted of collecting the biocontrol agents in southeastern states and releasing them at selected sites in California. Sites were to be selected in the Delta and also in upstream portions of the San Joaquin River System. As release efforts were being conducted, data were regularly collected to determine the seasonal growth characteristics of the waterhyacinth populations at each site. After establishment of the biocontrol agents was verified at a site, data were also collected to evaluate the control effectiveness of the biocontrol agents.

Nursery site selection

29. The primary criterion in evaluating nursery sites was the requirement that the waterhyacinth population at the site would remain in place for a sufficient period of time to allow build-up of the biocontrol agents. Selected sites, therefore, had to be located in areas which would not require treatment and which would not be influenced by high water levels. For nursery sites in the Delta, a second criterion was the overall geometric pattern created by the locations of the selected sites. This criterion was established because it was assumed that more widespread dispersion of the biocontrol agents from the sites would occur if the sites were located in different portions of the Delta.

30. In all, four nursery sites were chosen in the Delta. The locations of these sites are illustrated in Figure 3. Detailed descriptions of work conducted at each of these sites are provided in the following major sections of this report. Sites within Salt Slough were chosen as upstream locations for establishment of the biocontrol agents. Surveys conducted in December 1982 identified this slough as an uppermost source of waterhyacinth in the San Joaquin River System.

Postponement of upstream efforts

31. In 1983, prior to initial releases of any bicontrol agents in Salt Slough, the CDBW indicated that its authority for waterhyacinth control was limited to areas within the legal boundaries of the Delta. Because the biological control project was being conducted under CDBW authority, efforts to

establish founder colonies in upstream portions of the San Joaquin River System were indefinitely postponed.

Old River Field Test

Purpose

32. The intended purpose of efforts at this site was the establishment of *N. bruchi* and *S. albiguttalis* founder colonies in the Delta and the evaluation of the control effectiveness of this combination of control agents.

Site description

33. The Old River Site is located in the southern portion of the Delta (Figure 3). The site is approximately 1.6 km west of Tracy Road within a natural, closed-end extension of Old River. The site (Figure 4) is 30.5 m wide by 122 m long, and average water depth ranges from about 0.6 m at

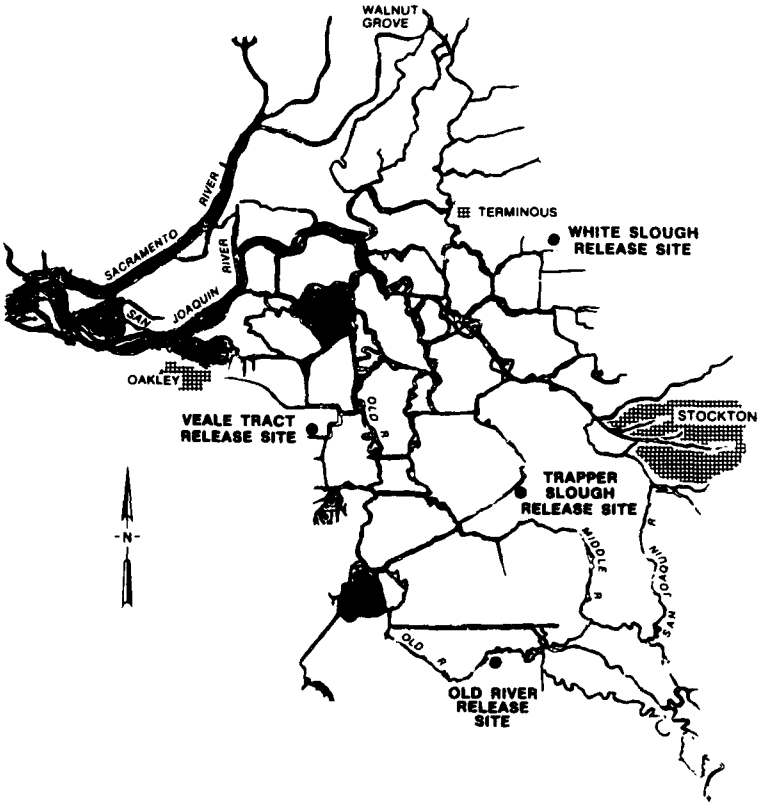


Figure 3. Map of the Delta showing locations of the four field sites

Figure 4. The Old River field site

the closed end to about 1.5 m at the open end. A floating boom was placed across the open end to limit the loss of waterhyacinth plants from the site during high water.

Materials and methods

34. *Neochetina bruchi* releases. Releases of *N. bruchi* were initiated at the Old River Site in 1982 (Cofrancesco 1984). Releases in 1982 consisted of 260 adults in July and 200 adults in October. In 1983, releases consisted of 290, 300, and 190 adults in April, July, and August, respectively. Releases in 1984 consisted of 124 adults in June and 50 adults in August.

35. *Sameodes* releases. The initial *Sameodes* release at the Old River Site occurred in August 1983 and consisted of 200 larvae (Cofrancesco 1984). In July 1984, two releases were made totaling 2,550 larvae. In August 1984, an additional 780 larvae were released. In 1985, *Sameodes* releases were accomplished by transplanting infested plants from the BCSP greenhouse colony. No larvae counts are available for these releases. Removing portions of the greenhouse colony also helped keep the size of the colony at a manageable level. Though exact dates were not recorded, the majority of the releases were made during spring and fall.

36. Sampling schedule. Sampling at the Old River Site was conducted in October and December 1982; in April, July, and October 1983; and in June, August, and October 1984 and 1985.

37. Sampling procedure: 1982-1983. On each sampling date in 1982 and 1983, five points were randomly selected within the Old River Site. Each point served as the center of a circular (7.6 m radius) sampling plot. Locations for three 0.25-sq m samples were identified within each of the five circular areas by randomly selecting three compass headings (1 to 360) and a corresponding distance value (0.3 to 7.6 m) along each compass heading from the center point. This procedure provided locations for fifteen 0.25-sq m samples at the site on each date. Styrofoam watershoes were worn to maneuver atop the waterhyacinth mat with minimal disturbance of the plants.

38. Data recorded for the waterhyacinth population included percent cover of the entire site (estimated by three observers), sample height, sample density (mature plants), sample weight (wet), and daughter plant (DP) density. Offshoots via asexual reproduction were considered daughter plants if they were still connected to a mature plant and had not yet developed an extensive root system. Sample height (centimetres) was determined by measuring the length of the first mature leaf of one plant from each sample. Sample density and DP density were determined by separating daughter plants from mature plants and counting the total numbers of each per sample. Sample weight was measured to the nearest ounce and later converted to kilograms.

39. Sampled plot data recorded for the biocontrol agents from each 0.25-sq m sample included the following. For *N. bruchi*, numbers of adults and larvae were counted from each plant. Additionally, the total number of plants with observable larval damage were recorded, even if larvae were not found in the plant. For *Sameodes*, each plant was examined for larvae and pupae and their numbers were recorded.

40. Sampling procedure: 1984-1985. During 1984 and 1985, the sampling procedure was modified to account for an observable lack of uniformity in the *N. bruchi* population within the site. The site was divided along its length into six sampling areas (15.2 m length × 30.4 m width). A circular sampling plot (7.6-m radius) was established around a randomly selected point within each sampling area. As described in paragraph 36, three samples were taken from each sampling plot. This procedure provided a stratified random sampling design with eighteen 0.25-sq m samples taken on each date. In addition to

the increase in sample number, the procedure was also modified to include height measurements from five plants per sample instead of from one plant per sample. The procedure for recording measurements of all other parameters remained the same.

41. Data analysis. Percent cover estimates of the three observers for each sampling date were averaged. Mean values of all other plant parameters were obtained for the entire site. An analysis of variance (ANOVA) was used to determine if mean values varied significantly among sampling dates. Due to differences in sample numbers between dates, the GLM procedure as provided by the Statistical Analysis System (1982) was used.

42. Mean numbers of each life stage of *N. bruchi* and *Sameodes* were calculated at the sample level by averaging the cumulative totals for all plants from each sample. Additionally, sample totals were divided by respective sample densities (plants) and these values were averaged to provide means for the number of insects per plant.

Results

43. Waterhyacinth population. Mean values of all plant growth parameters for the Old River Site are given in Table 1. In 1982, surface coverage of the site by waterhyacinth was 100 percent in both October and December. Sample density increased from 11.9 plants/sample to 22.8 plants/sample. Sample weight also increased from 8.7 kg/sample in October to 12.9 kg/sample in December. Though sample density and weight increased, individual plant size decreased from October to December. Mean plant height decreased from 110.9 cm in October to 99.9 cm in December. Reductions in average plant weight showed a similar trend. Though DP density was higher in December, the mean numbers of daughter plants per mature plant were similar on both dates.

44. In 1983, surface coverage of the site by waterhyacinth increased from 30 percent in April to 98 percent and 100 percent in July and October, respectively. Plant density increased to 29.5 plants/sample in July, and then declined prior to the October sampling date. Sample weight, however, was highest in October at 9.9 kg/sample. Though fewer in number, individual plants were larger in October than in July. Mean plant height values were 49.5 cm in July and 105.8 cm in October. During this period, individual plant weights also increased from 0.22 kg/plant to 0.58 kg/plant. The daughter plant density was highest in April and decreased throughout the growing season.

45. In 1984, surface coverage of the site was 80 percent in June and 100 percent in August and October. Sample density decreased from 26.8 plants/sample in June to 13.9 plants/sample in August, and then increased to 15.6 plants/sample in October. Maximum sample weight was 8.7 kg/sample in October. Plant height values ranged from 20.3 cm in June to 101.7 cm in August. Though there was a slight reduction in plant height between August and October, the average weight of individual plants increase from 0.51 kg/plant in August to 0.56 kg/plant in October. Daughter plant density at the site decreased from 26.2 daughters/sample in June to 6.3 daughters/sample in both August and October.

46. In 1985, surface coverage of the site was 10 percent in June, 60 percent in August, and 80 percent in October. Average plant size in October 1985 was significantly less than in previous years. In October 1985, mean values for plant height and plant weight were 33.5 cm and 0.25 kg/plant, respectively. Sample weight (3.3 kg) was also significantly less than in previous years. Year-end daughter plant production, conversely, was significantly higher in 1985. This increase apparently reflects the continued presence of open water within the site for continued waterhyacinth colonization, a factor which did not exist at the end of previous growing seasons.

47. Biocontrol agent populations. Results for numbers of *N. bruchi* collected at the Old River Site are presented in Table 2. Initial establishment of the *N. bruchi* population at the site was verified in December 1982 by the collection of 12 larvae from samples. Fresh feeding damage by adult weevils was also observed near the point of the October release, but no adults were collected in samples.

48. No damage indicative of *N. bruchi* was observed at the site in April 1983, nor were adults or larvae collected in samples. In July 1983, adult weevil feeding damage was observed, but no adults or larvae were collected. This observation of adult feeding damage did not necessarily verify successful overwintering by *N. bruchi* since weevils had been released at the site in April (see paragraph 33). In October 1983, 8 adults and 87 larvae were collected from the samples. Adult feeding damage was observed on plants throughout the site.

49. The *N. bruchi* population survived the 1983-1984 winter. Totals of 2 adults and 40 larvae were collected in June 1984. Though sparse, the weevil population seemed established throughout the site. In August, totals of

47 adults and 692 larvae were collected from samples. The density of *N. bruchi* in plants sampled was 0.17 adult/plant and 2.48 larvae/plant. Fifty-five percent of the sampled plants had been damaged by larvae. In October 1984, the *N. bruchi* population had increased to 0.52 adult/plant and 3.03 larvae/plant. Larval feeding damage was observed in 68 percent of the sampled plants.

50. Successful overwintering was again verified in June 1985. Totals of 13 adults (0.09 adult/plant) and 163 larvae (1.15 larvae/plant) were collected in samples. Larval damage occurred in 65 percent of the sampled plants. In August 1985, numbers of *N. bruchi* averaged 0.42 adult/plant and 3.06 larvae/plant. Ninety-three percent of the sampled plants had larval damage. By October 1985, the *N. bruchi* population averaged to 1.38 adults/plant and 5.92 larvae/plant. Larval damage was observed in every sampled plant.

51. *Sameodes* was not successfully established at the site during the study. By booming the open end of the site, we created a confined area in which the waterhyacinth mat could expand. During 1983 and 1984, the waterhyacinth mat had completely covered the site before releases of *Sameodes* were made. Because the morphological type of waterhyacinth which *Sameodes* prefers is only found in open water, lack of establishment is due partly because releases were not made early enough in the growing season.

Discussion

52. The waterhyacinth population at the Old River Site showed seasonal changes in measured parameters typical of normal growth during 1982-1984. The seasonal pattern consisted of a change from numerous, small plants in the spring to fewer, larger plants in the fall. During 1983, results indicate that spring regrowth was initiated by April and that surface colonization continued into June. Seasonal increases in individual plant size, which was accelerated by complete colonization of the surface area (i.e., attainment of 100 percent surface coverage), included increases in both height and weight. This caused an increase in average sample weight, even though sample density decreased during the growing season.

53. In 1985, the magnitude of the seasonal increase in the waterhyacinth population was significantly less than in previous years, and complete surface colonization of the site did not occur (Table 1). When weighted by percent cover of the entire site (Figure 5), sample density (mature plants)

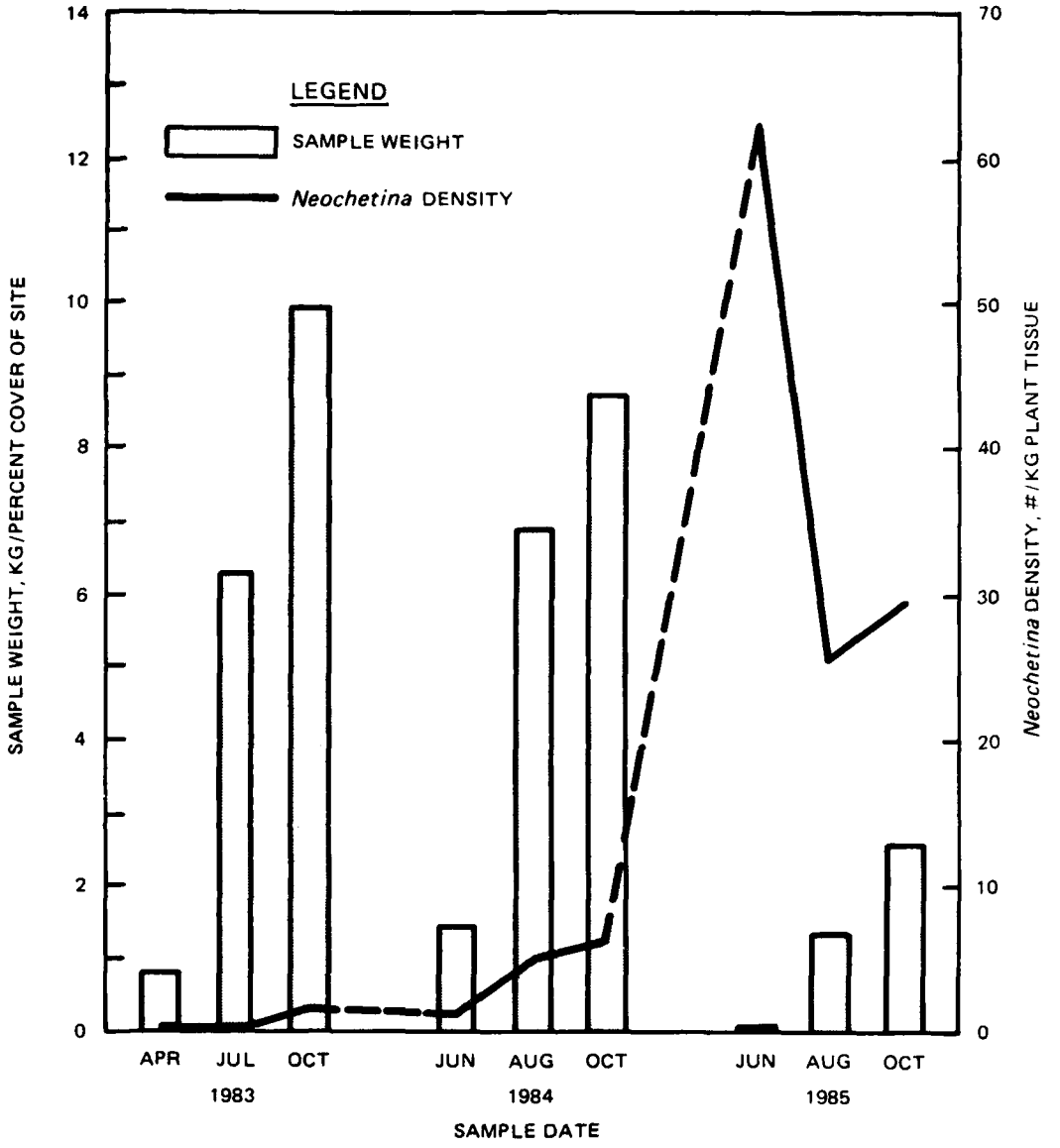


Figure 5. Annual trends in waterhyacinth standing crop (fresh weight) and *N. bruchi* density observed at the Old River field site. Standing crop expressed as mean sample weight, kg, multiplied by the percentage of the site covered by waterhyacinth. *N. bruchi* density expressed as mean number per sample divided by the mean sample weight (fresh weight). Dotted lines between years reflect unwillingness to extrapolate these relationships during winter months when cold temperatures caused heavy mortality to both plants and insects.

in October 1985 was 22 percent lower than the October 1984 value. The "weighted" daughter plant density in October 1985, however, was 3.8 times greater than in 1984. Though the total number of plants (i.e., the sum total

of mature plants and daughter plants) was higher at the site in 1985 than in 1984, mean sample weight (fresh) in 1985, when corrected for percent cover, actually showed a 66 percent reduction from the 1984 value. This reduction in standing crop was attributed to a 64 percent decrease in size (height) of individual mature plants at the site.

54. The observed decline in the waterhyacinth standing crop (fresh weight) coincided with a significant increase in the density of *N. bruchi* within the site. Though noticeable reductions in the *N. bruchi* population occurred during the 1984-1985 winter, density of the weevil population was greater than one individual per mature plant in June 1985 and increase approximately sixfold during the growing season (Table 2). More significantly, because the waterhyacinth standing crop was much reduced, the *N. bruchi* population had less plant material to inflict damage to in 1985. This ecological relationship is depicted in Figure 5, which shows the ratio of the number of *N. bruchi* per kilogram of fresh plant tissue.

White Slough Field Test

Purpose

55. The intended purpose of the study at this site was the establishment of *N. eichhorniae* and *S. albiguttalis* colonies in the Delta and the evaluation of the control effectiveness of this combination of biocontrol agents.

Site description

56. The site selected for this study is in the northeastern portion of the Delta (Figure 3). The site, located in the eastern end of White Slough, is a canal approximately 220 m long by 18 m wide (Figure 6). Average water depth at the center of the site is about 1.5 m. Effluent from a municipal sewage treatment facility enters the site from the eastern end. A floating boom was placed across the western end of the site to maintain a uniform waterhyacinth mat.

Materials and methods

57. *Neochetina eichhorniae* releases. *Neochetina eichhorniae* releases were initiated in April 1983 at White Slough (Cofrancesco 1984). In 1983, releases of *N. eichhorniae* consisted of 629 adults in April and 971 adults in July. In 1984, releases of adults totaled 500 in May, 869 in June, and 1,000 in August.

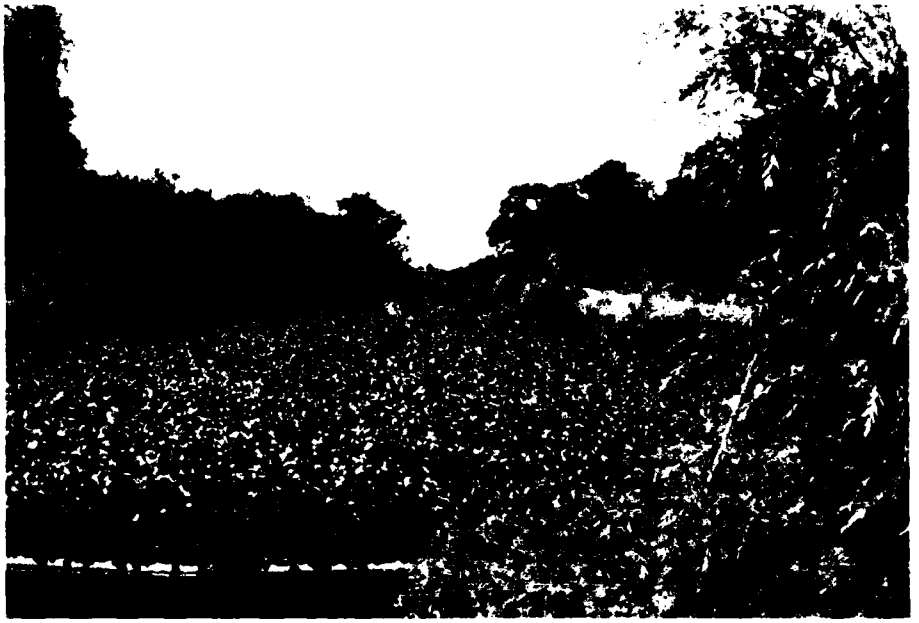


Figure 6. The White Slough field site

58. *Sameodes* releases. Releases of *Sameodes* were initiated at White Slough in 1984 (Stewart 1985). Three separate releases of *Sameodes* larvae were made at the site in August 1984 and totaled ca. 3,500 individuals. In 1985, infested plants from the greenhouse colony were released on various dates. Numbers included in these releases are not available.

59. Sampling schedule. Sampling at the White Slough Site was conducted in April, July, and October 1983; in June, August, and October 1984; and in June and August 1985.

60. Sampling procedure. The White Slough Site was sampled by the same procedure in 1983 as was described for the Old River Site in paragraphs 37-39. In 1984 and 1985, the procedure described for the Old River Site in paragraph 40 was used, except that dimensions of the six sampling areas were 36.6 m long by 18.3 m wide.

61. Data analysis. The data were analyzed by the same techniques as described for the Old River Site in paragraphs 41 and 42.

Results

62. Waterhyacinth population. Mean values for waterhyacinth growth parameters measured at the White Slough Site during this study are given in Table 3. During 1983, surface coverage of the site by waterhyacinth increased

from 80 percent in April to 100 percent in July and October. Sample density increased to 19.2 plants/sample in July and decreased to 8.1 plants/sample by October. Sample weight was also highest in July at 9.8 kg/sample. Individual plant size increased throughout the 1983 growing season. In October, average plant height and weight values were 122.7 cm and 0.67 kg/plant, respectively. Mean values for DP density decreased from 22.7 daughters/sample in April to 9.1 daughters/sample in October.

63. In 1984, surface coverage of the site by waterhyacinth was 100 percent on each sampling date. Sample density was 20.4 plants/sample in June and decreased to 11.3 plants/sample by August. Sample weight increased from 5.8 kg/sample in June to 7.0 kg/sample in October, but differences were not significant. Mean values for plant height and individual plant weight peaked in August at 112.4 cm and 0.57 kg/plant, respectively. Fewer daughter plants were present at the site in August than in June or October.

64. In 1985, surface coverage of the site by waterhyacinth was 100 percent on both dates. Sample density decreased from 36.3 plants/sample in June to 12.4 plants/sample in August. Sample weight, conversely, showed a significant increase between the June and August surveys. Mean values were 5.2 kg/sample and 7.1 kg/sample, respectively. Height and weight values for individual plants increased significantly between June and August, and were similar to values recorded in 1984. Daughter plant abundance was similar between dates.

65. Biocontrol agent populations. Results for numbers of *N. eichhorniae* collected at the White Slough Site are presented in Table 4. No damage indicative of *N. eichhorniae* were observed at the site in April 1983. In July 1983, adult feeding damage was observed in the eastern end of the site near the location of the April release (paragraph 57), but no damaged plants or insects were collected in samples. Feeding damage was more widespread within this same general area of the site in October 1983, but again no adults or larvae were collected.

66. In 1984, a stratified sampling design (paragraph 60) was adopted that resulted in a higher probability of sampling within the portion of the site where *N. eichhorniae* was established. In June 1984, adult weevil feeding scars were observed in Plots 1 through 4, with most intense feeding in Plots 1 and 2 (i.e., east end of site). In August 1984, plants with adult feeding scars were collected in Plots 1 thru 5. Adults and larvae were collected in

small numbers in samples from Plots 1 through 4. Overall, mean numbers of *N. eichhorniae* at the site were 0.17 adult/plant and 0.06 larva/plant. The occurrence of adults was highest in Plots 1 and 2, where adult density averaged about one adult weevil for every two plants. In October 1984, *N. eichhorniae* adult feeding scars were observed on plants in each plot. In total, 13 adults and 26 larvae were collected from samples. Adult weevils were most abundant in Plot 2, and larvae were collected almost exclusively from samples in Plots 1 and 2.

67. In June 1985, plants with adult feeding scars were present in samples from each plot. Twelve *N. eichhorniae* adults were collected from Plots 1, 2, and 3, and 118 larvae were collected in total from all plots. Twenty-four percent of the sampled plants had been damaged by larval feeding. By August 1985, the *N. eichhorniae* population had increased to an average abundance of 0.50 adult/plant and 0.63 larva/plant. Sixty-two percent of the plants sampled were damaged by larval feeding. In addition to *Neochetina*, two *S. albiguttalis* larvae were collected in a sample from Plot 1. *Sameodes albiguttalis* feeding damage was also observed in Plot 6, but no larvae were collected in samples from this plot.

Discussion

68. Waterhyacinth at the White Slough Site exhibited normal seasonal growth patterns during 1983-1985 (Figure 7). Spring regrowth at this site in 1983 was well under way by April, and complete surface colonization was accomplished at the site by June in subsequent years. Plant density peaked in June to July in each year, and subsequently declined in late summer and fall. In 1983, plant density declined sufficiently to allow renewed production of daughter plants during the end of the growing season.

69. The *N. eichhorniae* population was well established at the site by 1985. Initial establishment occurred in Plots 1 and 2. During 1984, the weevil population increased in abundance and dispersed from the original locus into the center portion of the site. With later releases of adults in Plot 6 in August 1984, *N. eichhorniae* colonized the entire site by August 1985, at which time 62 percent of all sampled plants had been damaged by larval feeding. Population levels of *N. eichhorniae* did not become high enough to affect reductions in the waterhyacinth population (Figure 7) during the period of this study.

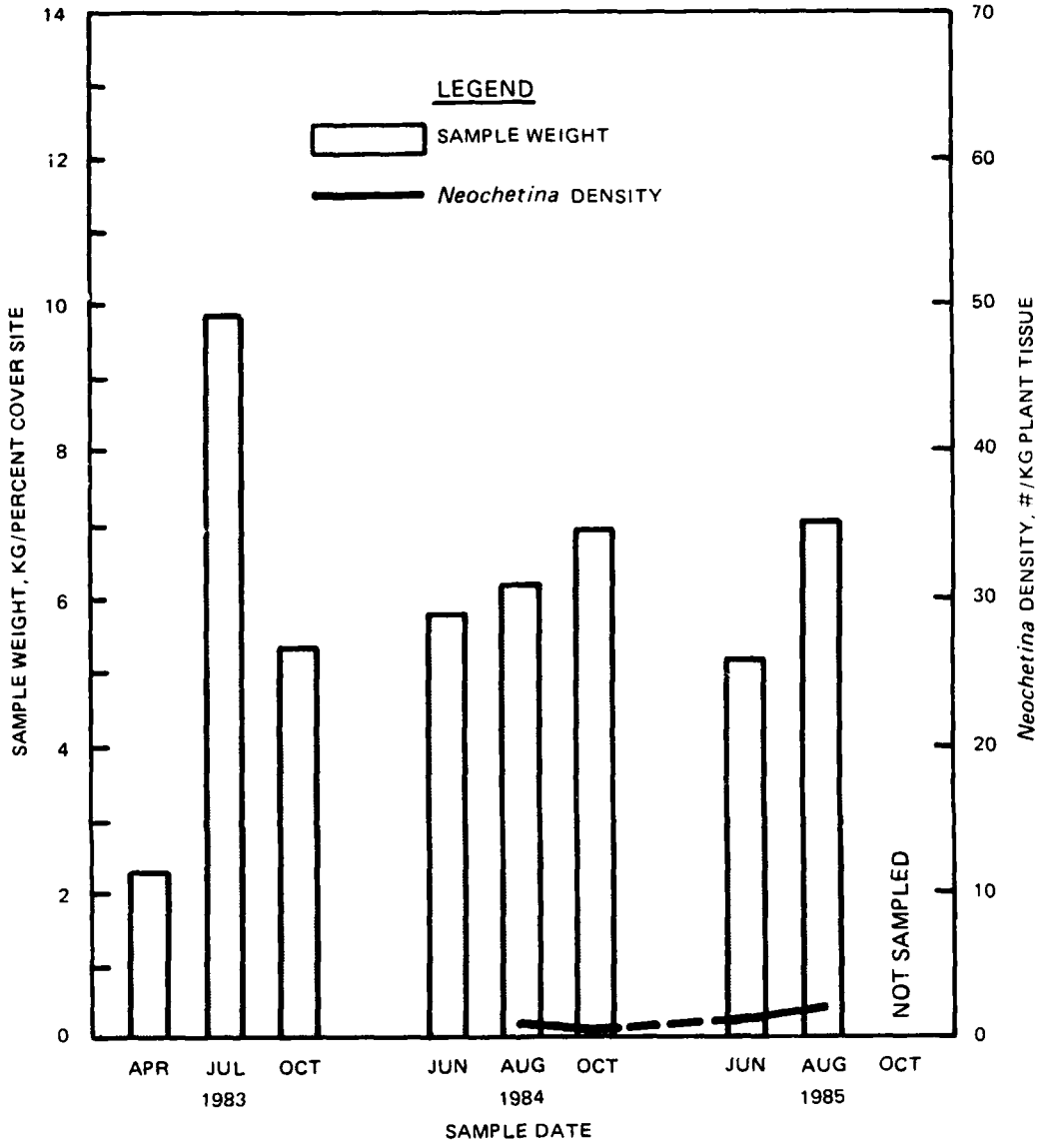


Figure 7. Annual trends in waterhyacinth standing crop (fresh weight)and *N. eichhorniae* density observed at the White Slough field site. Standing crop expressed as mean sample weight, kg, multiplied by the percentage of the site covered by waterhyacinth. *N. eichhorniae* density expressed as mean number per sample divided by the mean sample weight (fresh weight). Dotted lines between years reflect unwillingness to extrapolate these relationships during winter months when cold temperatures cause heavy mortality to both plants and insects

70. A small colony of *S. albiguttalis* was also present at the site in August 1985. Since this was the last sampling date at this site, permanent establishment of this control agent species could not be confirmed.

Inadequate establishment of *Sameodes* at this site was attributed to failure to conduct releases when the preferred growth form of waterhyacinth was present (paragraph 51).

Trapper Slough Field Test

Purpose

71. The purpose of the test conducted at Trapper Slough was to establish and evaluate the efficacy of a combination of all three biocontrol agents in the Delta.

Site location and description

72. The Trapper Slough Site is located in the central portion of the Delta (Figure 3). The section of Trapper Slough included in the study begins at the intersection of State Highway 4 and Bacon Island Road and extends approximately 975 m to the southwest (Figure 8). The site is 30.5 m wide, and average water depth is about 1.8 to 2.4 m. Because of negligible water flow through the site, placement of restraining boom was not necessary.

Materials and methods

73. *Neochetina* releases. The initial release of *N. bruchi* was made in October 1982 (Cofrancesco 1984) and consisted of 575 adults. In April 1983, ca. 200 *N. eichhorniae* adults were released. A second 1983 release was made in July and consisted of ca. 275 *N. bruchi* adults.

74. *Sameodes* releases. Releases of approximately 450 first instar larvae were made in August 1983 (Cofrancesco 1984). In September 1983, four similar releases totaling 430 larvae were made. Five releases were made in October and totaled 975 larvae.

75. Sampling schedule. Sampling at Trapper Slough was conducted in October and December 1982; in April, July, and October 1983; and in June 1984.

76. Sampling procedure. The sampling procedure used during 1982 and 1983 was identical to that described for the Old River Site in paragraphs 37-39, except that *Neochetina* adults were separated to species at this site. In 1984, the sampling procedure was modified similarly to that described for the Old River Site in paragraph 40. Sampling area dimensions at the Trapper Slough Site however, were 160 m length by 30.5 m width.

77. Data analysis. The data were analyzed by the same procedures described in paragraphs 41-42 for the Old River Site.

Figure 8. The Trapper Slough field site

Results

78. Waterhyacinth population. Mean values of waterhyacinth growth parameters for the Trapper Slough Site are given in Table 5. In 1982, surface coverage of the site by waterhyacinth was 88 percent in October and 85 percent in December. Though density values of plants per sample were similar, sample weight increased from 4.5 kg/sample in October to 6.2 kg/sample in December. Individual plants showed an increase in weight, but not in height. Mean height values were 17.1 cm in October and 16.5 cm in December. Mean weight values for these dates were 0.13 kg/plant and 0.17 kg/plant, respectively. Daughter plants were present in low numbers on both dates.

79. In 1983, surface coverage of the site by waterhyacinth increased from 60 percent in April to 85 percent in October. Sample density showed little change during the growing season. The highest mean value (34.6 plants/sample) was from July samples. Maximum plant height at the site was 15.1 cm in October. Mean sample weights were 5.5 kg/sample in April, 4.3 kg/sample in July, and 4.4 kg/sample in October. Average weights for individual plants followed this general pattern. Daughter plants were about 8 to 10 times more abundant at the site in April than in July or August.

80. In June 1984, surface coverage of the site was 86 percent. Sample density and sample weight were 37.9 plants/sample and 5.3 kg/sample, respectively. The mean height and weight of individual plants were 9.7 cm and 0.14 kg/plant, respectively. Daughter plant abundance at the site in June 1984 was 7.6 daughters/sample.

81. Biocontrol agent populations. Establishment of the biocontrol agents was never documented at the Trapper Slough Site. No individuals of any of the three species were collected from the samples, nor was feeding damage ever observed on sampled plants. Furthermore, in August 1984, two parallel transects extending the length of the site were surveyed to determine if isolated colonies of the biocontrol agents were established in the site. No positive sightings indicative of recent biocontrol agent feeding were observed during these surveys. The site, therefore, was excluded from subsequent sampling efforts.

Discussion

82. The waterhyacinth population at the Trapper Slough Site did not show seasonal changes in the measured parameters. In general appearance, individual plants were chlorotic and never showed significant increases in size. Maximum plant height at this site was approximately 80 percent less than observed at the other sites. This lack of vertical growth allowed a population of *Ludwigia* sp. to remain established within the waterhyacinth mat throughout the period of this study. The reduced waterhyacinth productivity at this site, in comparison to other sites, was attributed to low nutrient levels. Plants removed from this site and placed in tubs with minimal nutrient additives quickly exhibited healthy development.

83. Lack of establishment of the biocontrol agents at the Trapper Slough Site is believed to be directly related to the quality of the waterhyacinth population within the site. It is possible that the Trapper Slough plants offered an inadequate nutritional source for the insects, as well as a toughened substrate (cuticular layer) for oviposition and early instar penetration (Wright and Bourne 1986).

Veale Tract Field Test

Purpose

84. The purpose of the test conducted at the Veale Tract Site was to establish and evaluate the control efficacy of a combination of all three biocontrol agent species. This test was initiated because biocontrol agent populations had not been established at the Trapper Slough Site.

Site location and description

85. The Veale Tract Site is located in the northwestern portion of the Delta (Figure 3). The site lies within a man-made canal which projects westward from the north-south waterway connecting Rock Slough and Indian Slough. A 146 m section (Figure 9) of this canal was boomed-off to maintain the integrity of the waterhyacinth mat. This section of the canal is approximately 36.5 m wide. Water depth in the center of the site was about 1.8 to 2.4 m.

Materials and methods

86. *Neochetina* releases. Initial releases of *Neochetina* adults at the Veale Tract Site were made in October 1983 (Stewart 1985). These releases totaled 559 *N. bruchi* and 1,581 *N. eichhorniae*. In 1984, *N. eichhorniae* releases totaled 750 adults in June and 1,000 adults in August. No releases of *N. bruchi* were made in 1984. Total releases of *N. bruchi* were less than for *N. eichhorniae* because of lower population levels of *N. bruchi* in Texas (see paragraph 23).

87. *Sameodes* releases. The initial release of *Sameodes* was made in October 1983 (Stewart 1985), and totaled ca. 300 first instar larvae. In July 1984, an additional 3,200 larvae were released. As at other sites, introductions of plants infested with *Sameodes* were made at various times throughout the 1985 growing season.

88. Sampling schedule. Sampling at the Veale Tract Site was conducted in October 1983; in June, August, and October 1984; and in June and August 1985.

89. Sampling procedure. The procedure used at the Veale Tract Site in October 1983 was identical to that described for the Old River Site in paragraphs 37-39, except that *Neochetina* adults were separated to species. In 1984, the sampling procedure was modified similarly to that described for the

Figure 9. The Veale Tract field site

Old River Site in paragraph 40. Sample area dimensions at Veale Tract were 15.2 m length by 36.5 m width.

90. Data analysis. The data were analyzed by the same procedures as described in paragraphs 41-42.

Results

91. Waterhyacinth population. Mean values of all waterhyacinth growth parameters for the Veale Tract Site are given in Table 6. In October 1983, surface coverage of the site by waterhyacinth was 100 percent. Sample density was 11.5 plants/sample and sample weight was 4.3 kg/sample. For individual plants, mean values for height and weight were 75.6 cm and 0.39 kg/plant, respectively. Daughter plant production at the site in October was 12.4 daughters/sample.

92. In 1984, surface coverage of the site by waterhyacinth was 100 percent on each sampling date. Plant density was 38.3 plants/sample in June, and then decreased by approximately 75 percent to 10.5 and 11.3 plants/sample in August and October, respectively. Sample weight was significantly higher after June, this variable being affected by increases in the average size of individual plants. Plant height was greatest in August at 121.8 cm.

93. In June 1985, only one sample was collected from the Veale Tract Site. Though overall surface coverage of the site by waterhyacinth was 100 percent, informal surveys of the site that had been conducted during the 1984-1985 winter by BCSP personnel documented the complete fallout of the waterhyacinth mat. This fallout was attributed to severe frost damage to the plants. The sampling effort was reduced to one sample because this fallout was assumed to have resulted in high mortality in the biocontrol agent populations within the site. In the single sample taken, totals of 43 mature plants and 18 daughter plants were counted. In general, plant size was ca. one-half that recorded in June 1984. Average plant height was 23 cm and average plant weight was 0.06 kg. By August 1985, the waterhyacinth population had recovered. From nine samples taken at the site (i.e., 3 each from Plots 3, 4, 5), mean sample density and weight were 10.6 plants/sample and 5.4 kg/sample. Mean values for individual plant size were 105.9 cm and 0.52 kg.

94. Biocontrol agent populations. In June 1984, *Neochetina* adult feeding scars were observed on seven of the plants sampled from Plot 2. Adult feeding damage was also present in other areas of the site, but damaged plants were not collected from other samples, nor were any adults or larvae collected. In August 1984, plants with adult feeding damage were collected in Plots 3 and 4, and totals of 3 *Neochetina* adults (2 *N. bruchi* and 1 *N. eichhorniae*) and 11 *Neochetina* larvae were collected in Plot 3. Additionally, a single *S. albiguttalis* larva was collected from Plot 1. In October 1984, *Neochetina* adult feeding damage was found on plants from Plots 2, 3, and 6. Most intensive damage was observed in Plot 3, and totals of 3 *N. bruchi* adults and 15 *Neochetina* larvae were collected in samples from this plot. No *S. albiguttalis* were collected from the site on this trip.

95. Cold winter temperatures during the 1984-85 winter caused heavy mortality to the biocontrol agent populations. No individuals of any of the three species were collected in the June sample. Further, visual surveys of the entire site detected no evidence of the control agents. In August 1985, observations of *Neochetina* adult feeding scars within Plots 3 and 5 verified that at least one of the weevil species had survived the winter. In total, one adult weevil, later identified as *N. bruchi*, and four larvae were collected in Plot 5. No *Sameodes* were collected in samples, nor were signs of this species observed.

Discussion

96. The waterhyacinth populations at the Veale Tract Site exhibited normal growth patterns during the three year period of this study. Surface coverage of the site by waterhyacinth was 100 percent on each sampling date. Though average plant height was less than 1 m in October 1983, plants were healthy and no signs of insect damage or disease were observed. In 1985, waterhyacinth completely reestablished itself at the site following a hard freeze. This sequence of events demonstrated the weed's characteristic ability to overcome environmental stress. Though temperature data were not taken at the sites during winter months, observations made by BCSP personnel and local residents indicate that the water surface at Veale Tract froze. Similar occurrences were not reported at the other sites.

97. The effect of the 1984-1985 winter on the biocontrol agent populations was much more evident and longer sustained. Prior to the winter, *Neochetina* spp. predominantly *N. bruchi*, had made significant increases in population size, and weevil damage was widespread within the site. In 1985, the continued presence of *N. eichhorniae* and *S. albiguttalis* was not verified. *Neochetina bruchi* survival was verified in August, but population levels were much reduced from 1984 levels.

PART IV: DISPERSION OF BIOCONTROL AGENTS

Overall Test Design

98. Dispersion of the biocontrol agents from the four release sites to other waterhyacinth infestations was monitored to determine the ability of the biocontrol agents to naturally disperse throughout the Delta. Natural dispersion by the biocontrol agent species to additional sites is known to occur through two general methods, which are referred to herein as passive and active dispersion. Passive dispersion occurs when a waterhyacinth plant that contains a control agent is carried by wind or water currents to a new location. This type of dispersion includes all life stage of the insects. Active dispersion from site to site occurs as the result of flight, and is therefore limited to the adult stage. *Sameodes* adults are capable of active dispersal throughout their adult life period. In comparison, *Neochetina* adults do not always have functional wings and consequently can not always accomplish active dispersal. At present, researchers believe that wing muscle development is initiated by high temperatures, though earlier theories (e.g., unsuitable food, high weevil density, etc.) have not been completely discounted. Both types of dispersion from the release sites were noted and separately monitored.

Materials and Methods

Passive dispersion

99. Passive dispersion of the biocontrol agents from the four release sites was monitored by visual surveys for biocontrol agent feeding damage within waterhyacinth infestations in waterways connected to the release sites.

Active dispersion

100. Active dispersion of the biocontrol agents was monitored by operating light traps at night and by visual surveys. The light traps (Figure 10) consisted of four side-mounted, 15-watt black light bulbs and a top-mounted 175-watt mercury vapor bulb. This light system was mounted above a 36-in.-diam funnel, the mouth of which opened into a wood cabinet containing a series of collecting trays. This type of trap had been proven effective in collecting each of the three biocontrol agent species in Louisiana. Visual surveys

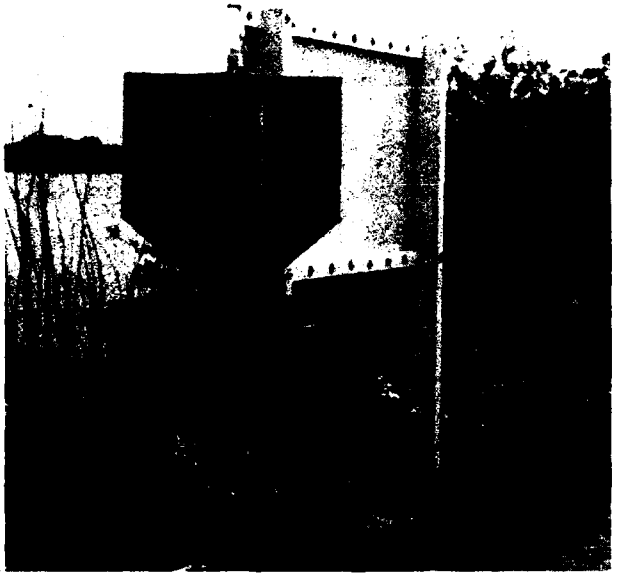


Figure 10. Light trap used to monitor biocontrol agent dispersion

for active dispersion were conducted in waterhyacinth infestations throughout the Delta, but particularly in privately owned irrigation ditches that had no direct connection to main waterways. These criteria were included to prevent confusion with passive dispersion.

Results

101. Passive dispersion of biocontrol agents from the release sites was not documented during this study. On no occasion was feeding damage by biocontrol agents observed outside the release sites. Passive dispersion, in fact, was prevented as a direct consequence of an extremely effective chemical control effort in the Delta. To prevent reinfestation of waterways essentially cleared of waterhyacinth by chemical control, applicators routinely sprayed the outer edges of the waterhyacinth mats at the release sites with 2,4-D (Figure 11). Since plants were prevented from "escaping" the sites, passive dispersion was also prevented.

102. Active dispersion was also shown to be extremely ineffective in the Delta during this study. No biocontrol agents were collected by light traps until August 1985 when 23 *N. eichhorniae* were recovered from the trap operated at the White Slough Site. Furthermore, no signs of biocontrol agent

Figure 11. Herbicide damage to the waterhyacinth mat at the boomed end of the Old River field site

feeding were observed in irrigation ditches or other nonconnected waterways in the Delta.

Discussion

103. Dispersion of the biocontrol agents from the four release sites was monitored to determine if they would naturally disperse to waterhyacinth throughout the Delta. Results demonstrated that, under the circumstances, passive dispersion had little chance of occurring. Active dispersion of adult insects was demonstrated for *N. eichhorniae*, but the successful establishment of dispersing populations outside the study sites was not verified. Furthermore, by 1984 the effectiveness of the chemical control effort was so widespread within the waterways that few permanent waterhyacinth populations remained in the Delta in which dispersing biocontrol agent colonies could become established.

PART V: SUMMARY AND CONCLUSIONS

Summary

104. Biocontrol agents were introduced into California to complement chemical control operations for waterhyacinth in the California Delta. Biocontrol agents introduced at four sites included two weevil species, *Neochetina bruchi* Hustache and *N. eichhorniae* Warner, and a pyralid moth species, *Sameodes albiguttalis* Warren. Colonies established at these sites were intended to function as nurseries for natural dispersion of these control agents throughout waterhyacinth infestations in the Delta.

105. Different combinations of the biocontrol agents were released at the four sites to allow comparisons of the effectiveness of the different species. At the Old River Site, *N. bruchi* and *S. albiguttalis* were released. At the White Slough Site, *N. eichhorniae* and *S. albiguttalis* were released. All three biocontrol agent species were released at the Trapper Slough and Veale Tract sites.

106. *Neochetina bruchi* was successfully established at the Old River Site by 1983. Results indicate that the continual yearly increase in the population size of *N. bruchi* was a major factor leading to the observed decline in the waterhyacinth population at this site in 1985. The occurrence of this decline was illustrated by marked seasonal decreases in both individual plant size and in population growth characteristics. *Sameodes albiguttalis* did not become established at this site.

107. *Neochetina eichhorniae* and *S. albiguttalis* were established at the White Slough Site. The *N. eichhorniae* population was present throughout the site by August 1985. The *S. albiguttalis* population was limited to two isolated colonies at either end of the site. Populations of these biocontrol agents at this site did not obtain sufficient size to demonstrate control effectiveness by the end of the study.

108. Establishment of all three species was verified at the Veale Tract Site in 1984. The *N. bruchi* population increased in size more rapidly than the *N. eichhorniae* population. Populations of these control agents, however, did not attain sufficient size to effect reductions in the waterhyacinth population. The 1984 through 1985 winter resulted in high mortality to the biocontrol agents. Successful overwintering was verified only for *N. bruchi*.

109. None of the biocontrol agents was established at the Trapper Slough Site, and *Sameodes* was not established in high numbers at any of the sites. At Trapper Slough, lack of establishment was attributed to poor plant quality resulting from low nutrient availability to the plants.

110. By comparing results from the Old River, White Slough, and Veale Tract sites it was evident that efforts to establish *N. bruchi* and *N. eichhorniae* were more successful than efforts with *S. albiguttalis*. Furthermore, *N. bruchi* populations increased in size more rapidly at the Old River Site than *N. eichhorniae* populations at the White Slough Site. Observations from the Veale Tract Site following the 1984-1985 winter suggest that *N. bruchi* was more tolerant of winter conditions than *N. eichhorniae*. Deloach and Cordo (1976a) also reported higher tolerance to cold temperatures by *N. bruchi*. Additionally, seasonal shifts in abundance ratios of these two species at Wallisville, Texas, indicate that higher proportions of *N. bruchi* populations survive during the winter.

111. Successful establishment of new colonies of these biocontrol agents through natural dispersion was not documented. Observations indicate that the potential for this occurrence was indirectly limited by the extensive success of chemical control in the Delta. Passive dispersion was restricted by chemical treatment of the outer edges of the waterhyacinth mats at the release sites. Though active dispersion of adult *N. eichhorniae* was documented at White Slough, the probability that dispersing adults will find waterhyacinth populations to colonize is low.

Conclusions

112. General conclusions of the project were as follows:

a. All three biocontrol agents can be established in the Delta. *Neochetina* spp. are much more easily established than *Sameodes* in confined release sites, and a resident colony of the latter must be maintained from which to make repeated releases.

b. *Neochetina bruchi* populations developed more quickly than *N. eichhorniae* populations. This comparison contradicts results from field studies in Southeastern states, but supports laboratory data indicating a shorter generation time for *N. bruchi*.

c. The biocontrol agents will effect a reduction in waterhyacinth populations in the Delta if allowed sufficient time. A decline in the waterhyacinth population at the Old River Site was attributed, at least in part, to *N. bruchi*.

d. The ability of the biocontrol agents to successfully disperse from the release sites and establish new colonies remains uncertain. The success of chemical control has resulted in few remaining waterhyacinth infestations within the Delta waterways. This limits the ability of the biocontrol agents to disperse naturally.

e. The most effective application of these biocontrol agents would center on their establishment in waterhyacinth infestations which occur in upstream portions of the San Joaquin River System. In these areas, the biocontrol agents would be less subjected to impacts from chemical control operations and could reduce the productivity of these upstream waterhyacinth populations. Further, the control agents would accompany any waterhyacinths moving downstream. This type of application would complement rather than conflict with the chemical control program.

REFERENCES

Addor, E. E. 1977. "A Field Test of Selected Insects and Pathogens for Control of Waterhyacinth; Preliminary Results for the 1975-76 Season," Technical Report A-77-2, Report 1, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Bock, J. H. 1970. "An Ecological Study of *Eichhornia crassipes* with Special Emphasis on its Reproductive Biology," Ph.D. dissertation, University of California, Berkeley.

Center, T. D. 1981a. "Release and Establishment of *Sameodes albiguttalis* for Biological Control of Waterhyacinth," Technical Report A-81-3, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

_____. 1981b. "*Sameodes albiguttalis*, a New Biocontrol Agent for Waterhyacinth," Aquatics, Vol 3, pp 8-12.

_____. 1982a. "Distribution and Effects of *Sameodes* on Waterhyacinth," Proceedings, 16th Annual Meeting, Aquatic Plant Control Research Planning and Operations Review, Miscellaneous Paper A-82-3, US Army Engineer Waterways Experiment Station, Vicksburg, Miss., pp 120-140.

_____. 1982b. "The Waterhyacinth Weevils, *Neochetina eichhorniae* and *N. bruchi*," Aquatics, Vol 4, No. 2, pp 8-19.

Center, Ted D., and Durden, Willey C. 1984. "Studies on the Biological Control of Waterhyacinth with the Weevils *Neochetina eichhorniae* and *N. bruchi*." Proceedings, 18th Annual Meeting, Aquatic Plant Control Research Program, Miscellaneous Paper A-84-4, US Army Engineer Waterways Experiment Station, Vicksburg, Miss., pp 85-98.

Center, Ted D., Durden, Willey C., and Carman, Debra A. 1984. "Efficacy of *Sameodes albiguttalis* as a Biocontrol of Waterhyacinth," Technical Report A-84-2, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Cofrancesco, A. F., Jr. 1984. "Biological Control Activities in Texas and California," Proceedings, 18th Annual Meeting, Aquatic Plant Control Research Program, Miscellaneous Paper A-84-4, US Army Engineer Waterways Experiment Station, Vicksburg, Miss., pp 57-61.

_____. "Biology of Aquatic Plants in the Galveston District," Technical Report in preparation, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Cordo, H. A., and DeLoach, C. J. 1978. "Host Specificity of *Sameodes albiguttalis* in Argentina: A Biological Control Agent for Waterhyacinth," Environmental Entomology, Vol 7, pp 322-328.

DeLoach, C. J. 1975. "Identification and Biological Notes on the Species of *Neochetina* That Attack Pontederiaceae in Argentina (Coleptera:Curculionidae: Bagoini)," Coleopterist Bulletin, Vol 29, No. 4, pp 257-265.

_____. 1976. "*Neochetina bruchi*, a Biological Control Agent of Waterhyacinth: Host Specificity in Argentina," Annals of the Entomological Society of America, Vol 69, pp 635-42.

DeLoach, C. J., and Cordo, H. A. 1976a. "Life Cycle and Biology of *Neochetina bruchi*, a Weevil Attacking Waterhyacinth in Argentina, with Notes on *N. eichhorniae*," Annals of the Entomological Society of America, Vol 69, pp 643-52.

_____. 1976b. "Ecological Studies of *Neochetina bruchi* and *N. eichhorniae* on Waterhyacinth in Argentina," Journal of Aquatic Plant Management, Vol 14, pp 53-59.

_____. 1978. "Life History and Ecology of the Moth *Sameodes albiguttalis*, a Candidate for the Biological Control of Waterhyacinth," Environmental Entomology, Vol 7, pp 309-321.

Goyer, R. A., and Stark, J. D. 1984. "The Impact of *Neochetina eichhorniae* on Waterhyacinth in Southern Louisiana," Journal of Aquatic Plant Management, Vol 22, pp 57-61.

Holm, L. G., Plucknett, D. L., Pancho, J. V., and Herberger, J. P. 1977. The World's Worst Weeds: Distribution and Biology, University Press, Honolulu, Hawaii.

Manning, J. H. 1979. "Establishment of Waterhyacinth Weevil Populations in Louisiana," Journal of Aquatic Plant Management, Vol 17, pp 39-41.

O'Brien, C. W. 1976. "A Taxonomic Revision of the New World Subaquatic Genus *Neochetina* (Coleoptera:Curculionidae:Bagoini)," Annals of the Entomological Society of America, Vol 69, No. 2, pp 165-174.

Penfound, W. T., and Earle, T. T. 1948. "The Biology of the Waterhyacinth," Ecological Monographs, Vol 18, pp 448-472.

Perkins, B. D. 1973. "Release in the United States of *Neochetina eichhorniae* (Warner), an Enemy of Waterhyacinth," Proceedings of the 26th Annual Meeting of the Southern Weed Science Society, p 368 (Abstr).

Perkins, B. D., and Maddox, D. M. 1976. "Host Specificity of *Neochetina bruchi* Hustache (Coleoptera:Curculionidae): A Potential Biological Control Agent for Waterhyacinth," Journal of Aquatic Plant Management, Vol 14, pp 59-64.

Sanders, D. R., Sr., and Theriot, E. A. 1986. "Large-Scale Operations Management Test (LSOMT) of Insects and Pathogens for Control of Waterhyacinth in Louisiana; Volume II: Results for 1982-1983," Technical Report A-85-1, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Sanders, Dana R., Sr., Theriot, Edwin A., and Perfetti, Patricia. 1985. "Large-Scale Operations Management Test (LSOMT) of Insects and Pathogens for Control of Waterhyacinth in Louisiana; Volume I: Results for 1979-1981," Technical Report A-85-1, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

SAS Institute, Inc. 1982. SAS User's Guide: Statistics, 1982 ed., Cary, N. C.

Stewart, R. Michael. 1985. "Biocontrol of Waterhyacinth in the California Delta," Proceedings, 19th Annual Meeting, Aquatic Plant Control Research Program, Miscellaneous Paper A-85-4, US Army Engineer Waterways Experiment Station, Vicksburg, Miss., pp 110-112.

Wright, A. D., and Bourne, A. S. 1986. "Effect of Leaf Hardness on Penetration by *Sameodes albiguttalis*," Journal of Aquatic Plant Management, Vol 24, pp 90-91.

Table 1

Means* of Plant Growth Parameters at the Old River Site

Sample Date	Percent Cover	Sample Density No./0.25-sq-m	Sample Weight kg/0.25-sq-m	Height cm	Plant Weight kg/plant	DP Density No./0.25-sq-m
Oct 82	100	11.9 (±1.67)	8.7 (±0.99)	110.9 (±3.65)	0.77 (±0.09)	5.3 (±1.78)
Dec 82	100	22.8 (±2.46)	12.9 (±1.56)	99.9 (±6.60)	0.57 (±0.06)	9.1 (±3.61)
Apr 83	30	13.5 (±3.63)	2.4 (±0.29)	18.5 (±1.54)	0.21 (±0.03)	17.7 (±3.75)
Jul 83	98	29.5 (±3.15)	6.4 (±0.63)	49.5 (±5.57)	0.22 (±0.02)	12.9 (±2.53)
Oct 83	100	17.5 (±1.85)	9.9 (±0.79)	105.8 (±4.69)	0.58 (±0.04)	4.9 (±2.33)
Jun 84	80	26.8 (±3.45)	1.9 (±0.24)	20.3 (±1.17)	0.08 (±0.014)	26.2 (±4.57)
Aug 84	100	13.9 (±1.43)	6.9 (±0.47)	101.7 (±2.34)	0.51 (±0.044)	6.3 (±1.27)
Oct 84	100	15.6 (±1.73)	8.7 (±0.69)	94.2 (±4.45)	0.56 (±0.040)	6.3 (±1.83)
Jun 85	10	47.3 (±7.69)	1.0 (±0.15)	10.1 (±1.07)	0.02 (±0.002)	15.7 (±5.92)
Aug 85	60	18.7 (±2.77)	2.3 (±0.20)	26.1 (±0.78)	0.13 (±0.01)	18.7 (±2.14)
Oct 85	80	15.3 (±2.40)	3.3 (±0.21)	33.5 (±1.47)	0.25 (±0.03)	30.3 (±2.85)

* Mean values for 1982 and 1983 sampling dates are based on 15 samples; 1984 values are based on 18 samples; June 1985 values are based on 3 samples; August and October 1985 values are based on 8 and 6 samples, respectively. Values in parentheses are ± 2 standard errors of the mean.

Table 2

Numbers* of *Neochetina bruchi* at the Old River Site

Sample Date	Adults		Larvae		Damaged Plants (larvae)	
	No. Collected	No./ Plant	No. Collected	No./ Plant	No./ Sample	% of Total
Oct 82	0	0.00	0	0.00	0.0	0
Dec 82	0	0.00	12	0.00	0.9	0
Apr 83	0	0.00	0	0.00	0.0	0
Jul 83	0	0.00	0	0.00	0.0	0
Oct 83	8	0.03	87	0.33	5.9	4
Jun 84	2	0.00	40	0.08	1.9	7
Aug 84	47	0.17	692	2.48	8.6	55
Oct 84	130	0.52	759	3.03	9.4	68
Jun 85	13	0.09	163	1.15	30.7	65
Aug 85	142	0.42	1028	3.06	17.4	93
Oct 85	130	1.38	529	5.92	15.3	100

* Values for 1982 and 1983 sampling dates are based on 15 samples. 1984 values on 18 samples; June 1985, 3 samples; August 1985, 18 samples; October 1985, 6 samples.

Table 3

Means* of Plant Growth Parameters at the White Slough Site

Sample Date	Percent Cover	Sample Density No./0.25 sq m	Sample Weight kg/0.25 sq m	Height cm	Plant Weight kg/plant	DP Density No./0.25 sq m
Apr 83	80	15.4 (±1.68)	2.9 (±0.28)	35.8 (±6.31)	0.19 (±0.023)	22.7 (±2.90)
Jul 83	100	19.2 (±2.81)	9.8 (±0.90)	102.5 (±2.93)	0.53 (±0.050)	10.4 (±2.17)
Oct 83	100	8.1 (±0.80)	5.3 (±0.37)	122.7 (±5.77)	0.67 (±0.081)	9.1 (±1.90)
Jun 84	100	20.4 (±1.64)	5.8 (±0.64)	64.6 (±2.06)	0.29 (±0.032)	11.4 (±1.97)
Aug 84	100	11.3 (±1.11)	6.2 (±0.55)	112.4 (±2.11)	0.57 (±0.072)	6.8 (±1.93)
Oct 84	100	12.8 (±1.99)	7.0 (±0.73)	100.2 (±4.12)	0.56 (±0.065)	8.9 (±2.63)
Jun 85	100	36.3 (±4.97)	5.2 (±0.34)	50.4 (±1.80)	0.15 (±0.014)	7.7 (±1.72)
Aug 85	100	12.4 (±1.17)	7.1 (±0.57)	101.4 (±1.85)	0.59 (±0.056)	6.1 (±1.32)

* Mean values for 1983 sampling dates are based on 15 samples; 1984 and 1985 values are based on 18 samples. Values in parentheses are ± 2 standard errors of the mean.

Table 4

Numbers* of *Neochetina eichhorniae* at the White Slough Site

Sample Date	Adults		Larvae		Damaged Plants (larvae)	
	No. Collected	No./ Plant	No. Collected	No./ Plant	No./ Sample	% of Total
Apr 83	0	0.00	0	0.00	0.0	0
Jul 83	0	0.00	0	0.00	0.0	0
Oct 83	0	0.00	0	0.00	0.0	0
Jun 84	0	0.00	0	0.00	0.0	0
Aug 84	41	0.17	13	0.06	1.6	14
Oct 84	13	0.06	26	0.11	2.2	17
Jun 85	12	0.02	118	0.18	8.6	24
Aug 85	111	0.50	142	0.63	7.7	62

* Values for 1983 sampling dates are based on 15 samples; 1984 and 1985 values are based on 18 samples.

Table 5

Means* of Plant Growth Parameters at the Trapper Slough Site

Sample Date	Percent Cover	Sample Density No./0.25-sq-m	Sample Weight kg/0.25-sq-m	Height cm	Plant Weight kg/plant	DP Density No./0.25-sq-m
Oct 82	88	35.9 (±5.59)	4.5 (±0.56)	17.1 (±1.12)	0.13 (±0.017)	0.8 (±0.40)
Dec 82	85	37.7 (±4.28)	6.2 (±0.61)	16.5 (±1.03)	0.17 (±0.017)	1.7 (±1.06)
Apr 83	60	31.1 (±3.52)	5.5 (±0.44)	11.2 (±2.04)	0.18 (±0.016)	23.3 (±3.93)
Jul 83	63	34.6 (±4.40)	4.3 (±0.37)	9.8 (±0.73)	0.13 (±0.017)	2.6 (±1.03)
Oct 83	85	27.5 (±3.43)	4.4 (±0.49)	15.1 (±1.92)	0.16 (±0.015)	1.9 (±0.55)
Jun 84	86	37.8 (±3.60)	5.3 (±0.42)	9.6 (±0.38)	0.14 (±0.009)	7.6 (±2.58)

* Mean values for 1982 and 1983 sampling dates are based on 15 samples; 1984 values are based on 3 samples. Values in parentheses are ± 2 standard errors of the mean.

Table 6

Means* of Plant Growth Parameters at the Veale Tract Site

Sample Date	Percent Cover	Sample Density No./0.25 sq m	Sample Weight kg/0.25 sq m	Height cm	Plant Weight kg/plant	DP Density No./0.25 sq m
Oct 83	100	11.5 (±1.03)	4.3 (±0.56)	75.6 (±7.49)	0.39 (±0.06)	12.4 (±2.02)
Jun 84	100	38.6 (±4.23)	5.4 (±0.52)	42.1 (±1.55)	0.14 (±0.011)	9.2 (±1.88)
Aug 84	100	10.5 (±1.33)	7.6 (±0.72)	121.8 (±1.24)	0.76 (±0.081)	6.0 (±1.86)
Oct 84	100	11.3 (±1.41)	8.6 (±0.38)	113.4 (±3.19)	0.81 (±0.089)	9.4 (±1.95)
Jun 85	100	43	2.6	23.0	0.06	18
Aug 85	100	10.6 (±0.89)	5.4 (±0.50)	105.9 (±1.67)	0.52 (±0.043)	3.2 (±1.09)

* Mean values for October 1983 are based on 15 samples; 1984 values are based on 18 samples; June 1985 values on 1 sample; August 1985 values on 9 samples. Values in parentheses are ± 2 standard errors of the mean. These values were not available for June 1985 means due to sample size.